

## Oxidative damage to DNA during aging: 8-Hydroxy-2'-deoxyguanosine in rat organ DNA and urine

(cancer/mutation/endogenous DNA adducts/8-hydroxyguanine/oxygen radicals)

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**ABSTRACT** Oxidative damage to DNA is shown to be extensive and could be a major cause of the physiological changes associated with aging and the degenerative diseases related to aging such as cancer. The oxidized nucleoside, 8-hydroxy-2'-deoxyguanosine (oh<sup>8</sup>dG), one of the ≈20 known oxidative DNA damage products, has been measured in DNA isolated from various organs of Fischer 344 rats of different ages. oh<sup>8</sup>dG was present in the DNA isolated from all the organs studied: liver, brain, kidney, intestine, and testes. Steady-state levels of oh<sup>8</sup>dG ranged from 8 to 73 residues per 10<sup>6</sup> deoxyguanosine residues or 0.2–2.0 × 10<sup>5</sup> residues per cell. Levels of oh<sup>8</sup>dG in DNA increased with age in liver, kidney, and intestine but remained unchanged in brain and testes. The urinary excretion of oh<sup>8</sup>dG, which presumably reflects its repair from DNA by nuclease activity, decreased with age from 481 to 165 pmol per kg of body weight per day for urine obtained from 2-month- and 25-month-old rats, respectively. 8-Hydroxyguanine, the proposed repair product of a glycosylase activity, was also assayed in the urine. We estimate ≈9 × 10<sup>4</sup> oxidative hits to DNA per cell per day in the rat. The results suggest that the age-dependent accumulation of oh<sup>8</sup>dG residues observed in DNA from liver, kidney, and intestine is principally due to the slow loss of DNA nuclease activity; however, an increase in the rate of oxidative DNA damage cannot be ruled out.

The biochemical mechanisms of aging are under extensive investigation but remain poorly understood. Endogenous metabolic processes are implicated as important factors in aging by the impressive inverse correlation between life-span and species-specific metabolic rate (1).

The damage produced by endogenously produced oxygen radicals has been proposed to be a major contributor to aging and the degenerative diseases associated with it, such as cancer and heart disease (2–7). *In vivo*, oxygen radicals are mainly produced as by-products of normal metabolism (8) from phagocytic cells (9) and from lipid peroxidation (10). Numerous defense systems protect cellular macromolecules against oxidation; nevertheless, there is a high rate of damage to DNA (11), proteins (12), and lipids (10, 13). The steady-state level of oxidatively modified nucleosides in genomic and mitochondrial DNA in rats (11) and the release of these damage products in human and rodent urine (14–16) have been determined. Oxidative damage to DNA has been estimated as 10<sup>4</sup> hits per cell per day in humans and 1 order of magnitude higher in rodents (7, 14, 15).

Some evidence suggests that an increased production of reactive oxygen species and/or a decreased efficiency of antioxidant defense systems is associated with aging (17, 18). Endogenous oxidative damage to lipids (19) and proteins (12) has been reported to increase with age. Damage to DNA has

been reported to increase with age in rats fed diets deficient in vitamin E, but not in rats fed vitamin E-sufficient diets (20).

The purpose of this study was to evaluate endogenous oxidative damage to DNA as a function of aging. This damage was assessed by measuring the steady-state level of 8-hydroxy-2'-deoxyguanosine (oh<sup>8</sup>dG) in DNA isolated from various tissues of rat and comparing its excretion rate into urine. oh<sup>8</sup>dG in DNA has been proposed to be an index of oxidative damage (11, 21, 22) and its excretion into urine provides an estimate of its rate of repair from DNA (16, 23). Part of this work has been presented previously (24).

### MATERIALS AND METHODS

**Animals, Organ Isolation, and Urine Collection.** Male Fischer 344 rats of different ages were purchased from Harlan–Sprague–Dawley (Indianapolis, IN). The rats were allowed to acclimate for at least 10 days prior to initiating these studies. Since the estimated life-span for a similar colony of Fischer 344 rats was 23 months (25), the age of the animals studied ranged from 1 to 24 months. Rats were fed standard laboratory diets and water ad libitum and were maintained in a temperature- and photoperiod-controlled (12 hr/day) room. In some cases, a nucleic acid-free diet (Dyets 510007, Dyets, Bethlehem, PA), was provided as indicated. For urine collections, the rats were housed individually in metabolic cages (Nalgene) and the 24-hr urine outputs were collected over 3 consecutive days and stored at –20°C until analyzed. At the end of the final day of urine collection, the rats were decapitated and the organs were removed and frozen until the time of DNA extraction. As specified, different organs from rats of the same age group or one specific organ from rats of different age groups were assayed together.

**DNA Isolation and Enzymatic Hydrolysis.** DNA from different tissues was isolated as described (26). The use of high-purity distilled phenol (International Biotechnologies) was necessary to prevent artifactual formation of oh<sup>8</sup>dG (21). After isolation of DNA from tissues, samples containing 200–400 μg of DNA were resuspended in 200 μl of 20 mM sodium acetate (pH 4.8) and digested to nucleotides with 20 μg of nuclease P1 (Sigma) at 37°C for 30 min. Then, 20 μl of 1 M Tris-HCl (pH 7.4) was added to the samples and they were treated with 1.3 units of *Escherichia coli* alkaline phosphatase (Sigma) at 37°C for 60 min.

**Determination of oh<sup>8</sup>dG in DNA.** The amount of oh<sup>8</sup>dG present in DNA was measured by HPLC with electrochem-

Abbreviations: oh<sup>8</sup>dG, 8-hydroxy-2'-deoxyguanosine; oh<sup>8</sup>Gua, 8-hydroxyguanine.

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ical detection (11, 21, 27) using a Bioanalytical Systems (West Lafayette, IN) amperometric detector with a glassy-carbon working electrode and an Ag/AgCl reference electrode. The applied oxidation potential was 0.6 V. Levels of  $\text{oh}^8\text{dG}$  in the samples were expressed relative to the content of DNA detected by absorbance at 260 nm. The  $\text{oh}^8\text{dG}$  standard was prepared following the procedure described by Kasai and Nishimura (28). The amount of DNA was calculated by comparing the absorbance peak area of deoxyguanosine obtained from the enzymatic hydrolysate of the DNA sample to a calibration curve for deoxyguanosine obtained from known amounts of enzymatically hydrolyzed calf thymus DNA.

**Determination of  $\text{oh}^8\text{dG}$  and 8-Hydroxyguanine ( $\text{oh}^8\text{Gua}$ ) in Urine.** The amount of urinary  $\text{oh}^8\text{dG}$  detected in rats of different ages was measured as described (16). Values of  $\text{oh}^8\text{dG}$  were expressed per kg of body weight per day. To measure  $\text{oh}^8\text{Gua}$ , urine samples were preprocessed (M.K.S., P.D., and B.N.A., unpublished data) and passed through an anti- $\text{oh}^8\text{dG}$  antibody column, which recognizes  $\text{oh}^8\text{dG}$ , 8-hydroxyguanosine, and  $\text{oh}^8\text{Gua}$ .  $\text{oh}^8\text{Gua}$  was eluted and quantitated electrochemically after HPLC separation.

**Statistical Analysis.** Values in the text and table represent the means  $\pm$  SEM. Statistical significance (regression analysis) was calculated by routines available in STATVIEW 512+ (Brainpower, Calabasas, CA).

## RESULTS

The relatively low oxidation potential of  $\text{oh}^8\text{dG}$  (+0.6 V versus the Ag/AgCl electrode) allowed us to detect and quantitate this compound by electrochemical detection that is both more sensitive and more selective than optical methods (29). The identity of  $\text{oh}^8\text{dG}$  present in tissue DNA was confirmed by (i) coelution with authentic  $\text{oh}^8\text{dG}$ , (ii) comparing its electrochemical-response profile (hydrodynamic voltammogram) with authentic  $\text{oh}^8\text{dG}$ , and (iii) identification of  $\text{oh}^8\text{Gua}$  as its thermal- and acid-catalyzed product. A similar approach has been used to confirm the identity of urinary  $\text{oh}^8\text{dG}$  (16).

Since DNA could be oxidatively damaged during isolation from tissues, especially from those containing high concentrations of oxidants, it was important to test whether the levels of  $\text{oh}^8\text{dG}$  could arise as an artifact of sample preparation. To assess this possibility, DNA was isolated from rat liver under different experimental conditions and the levels of  $\text{oh}^8\text{dG}$  were measured. As shown in Table 1, saturation of liver homogenates with argon did not significantly reduce the steady-state level of  $\text{oh}^8\text{dG}$  in DNA hydrolysates when compared to samples isolated under the air-saturated control condition. Incubation of these homogenates in air for 1 hr at room

temperature or the addition of 2-mercaptoethanol (2%; vol/vol) had no effect on the steady-state level of  $\text{oh}^8\text{dG}$ . However, 2-mercaptoethanol, at the above concentration, inhibited completely the formation of  $\text{oh}^8\text{dG}$  induced by  $\text{H}_2\text{O}_2$ /ascorbate (Table 1). In contrast to a previous report (21), we did not find any increase in the steady-state level of  $\text{oh}^8\text{dG}$  in DNA upon its hydrolysis over different times (0–2 hr) in the presence of different amounts of nuclease P1 or alkaline phosphatase. The addition of 2-mercaptoethanol to the enzymatic hydrolysis reaction mixture did not have a significant effect on the content of  $\text{oh}^8\text{dG}$  in DNA. Together, these results indicate that  $\text{oh}^8\text{dG}$  is unlikely to arise as an artifact of tissue homogenization, DNA isolation, or DNA hydrolysis.

The variability of the  $\text{oh}^8\text{dG}$  content in the DNA isolated from a given organ of a determined age is primarily related to animal variation and not to the reproducibility of the assay. This was concluded from the consistency of the results obtained by two different protocols: (i) assaying different organs from rats of a given age group or (ii) assaying a specific organ from rats of different ages.

Since in mammals the aging process does not affect all organs at the same rate, the age-dependent steady-state levels of  $\text{oh}^8\text{dG}$  were investigated in DNA isolated from different organs.  $\text{oh}^8\text{dG}$  was present in the DNA of all tissues assayed: liver, kidney, intestine, brain, and testes at steady-state levels ranging from 6 to 55 fmol per  $\mu\text{g}$  of DNA (8–73  $\text{oh}^8\text{dG}$  per  $10^6$  deoxyguanosine residues). In three of the organs examined, the levels of  $\text{oh}^8\text{dG}$  in DNA increased with the age of the animal (Fig. 1). The steady-state level of  $\text{oh}^8\text{dG}$  in DNA isolated from the livers of 24-month-old rats was  $23.2 \pm 1.8$  fmol per  $\mu\text{g}$  of DNA, which represented an increase of 164% over the value observed in the 1-month-old rats ( $8.8 \pm 1.4$  fmol per  $\mu\text{g}$  of DNA) (Fig. 1). In DNA isolated from kidney (Fig. 1), the increase in the levels of  $\text{oh}^8\text{dG}$  over the same period was 150%, from  $15.0 \pm 1.0$  to  $37.5 \pm 3.2$  fmol per  $\mu\text{g}$  of DNA. In intestine (Fig. 1), the steady-state level of  $\text{oh}^8\text{dG}$  in DNA increased from  $11.0 \pm 0.6$  to  $16.7 \pm 1.1$  fmol per  $\mu\text{g}$  of DNA, comparing rats 1 and 24 months old, respectively. In brain and testes (Fig. 1), the levels of  $\text{oh}^8\text{dG}$  in DNA were  $13.1 \pm 2.5$  and  $13.2 \pm 0.9$  fmol per  $\mu\text{g}$  of DNA, respectively, and did not change with age.

The measurement of urinary  $\text{oh}^8\text{dG}$  provides an estimate of the rate of repair of this oxidatively modified base by a nuclease activity. Urinary levels of  $\text{oh}^8\text{dG}$  decreased as a function of rat age (Fig. 2). For 24-month-old rats, the average daily output of  $\text{oh}^8\text{dG}$  was  $165 \pm 66$  pmol $\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ , which represents  $\approx 33\%$  of the value observed for the 2-month-old rats ( $481 \pm 163$  pmol $\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ). The urinary excretion of  $\text{oh}^8\text{Gua}$ , which represents the removal of the oxidized base by a glycosylase activity, did not change with the age of the rats. For  $\text{oh}^8\text{Gua}$ , the age-averaged value obtained for the urine of 2-, 4-, 12-, and 24-month-old rats was  $17,262 \pm 15,857$  pmol $\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  ( $n = 12$ ). The estimated urinary excretion rates exhibited considerable age-independent scattering, ranging from 4155 to 50,021 pmol $\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ . In an earlier study, rats fed glucose for 4 days excreted 40% less thymine glycol and 12% less thymidine glycol in urine (14). This suggests that some of the urinary thymine glycol, but little if any of the thymidine glycol, measured by this previous assay was from the diet. Studies designed to assess the contribution of diet toward the urinary excretion rate of  $\text{oh}^8\text{dG}$  showed that diet is not a factor (16), but that with  $\text{oh}^8\text{Gua}$  it is. In the current study, rats were fed a nucleic acid-free diet for 1 week. At day 7, the urinary excretion rate of  $\text{oh}^8\text{Gua}$  was compared to that of urine obtained on the day prior to subjecting the rats to this diet (day 0). A comparison of the rates of excretion indicate that the urinary level of  $\text{oh}^8\text{Gua}$  was 85% lower [ $3645 \pm 1166$  pmol $\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  (day 7)] than that obtained on the ad libitum diet [ $24,718 \pm 7481$  pmol $\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  (day 0)]. Thus,  $\text{oh}^8\text{Gua}$  is being excreted in the urine, presumably as a consequence of

Table 1. Effect of different experimental conditions on the content of  $\text{oh}^8\text{dG}$  in rat liver DNA

	$\text{oh}^8\text{dG}$ , fmol per $\mu\text{g}$ of DNA	
	No incubation	1 hr, 25°C
Air-saturated buffer (control)	21.3	20.9
Argon-saturated buffer	19.1	17.3
Control + 2-ME	22.7	18.6
+ $\text{H}_2\text{O}_2$ /ascorbate	ND	236*
+ $\text{H}_2\text{O}_2$ /ascorbate + 2-ME	ND	18.4

For the extraction of DNA, liver samples were homogenized in air- or argon-saturated 150 mM NaCl/1 mM EDTA/10 mM Tris-HCl buffer, pH 7.4, at 4°C. DNA samples were extracted immediately or after 1 hr of incubation at 25°C. 2-Mercaptoethanol (2-ME) (final concentration 2%; vol/vol) was added to the homogenates as indicated.  $\text{H}_2\text{O}_2$ /ascorbate is a mixture of 50 mM  $\text{H}_2\text{O}_2$ /20 mM ascorbic acid. ND, not determined. Values represent the average of two determinations.

\*Significantly different from control value;  $P < 0.01$ .

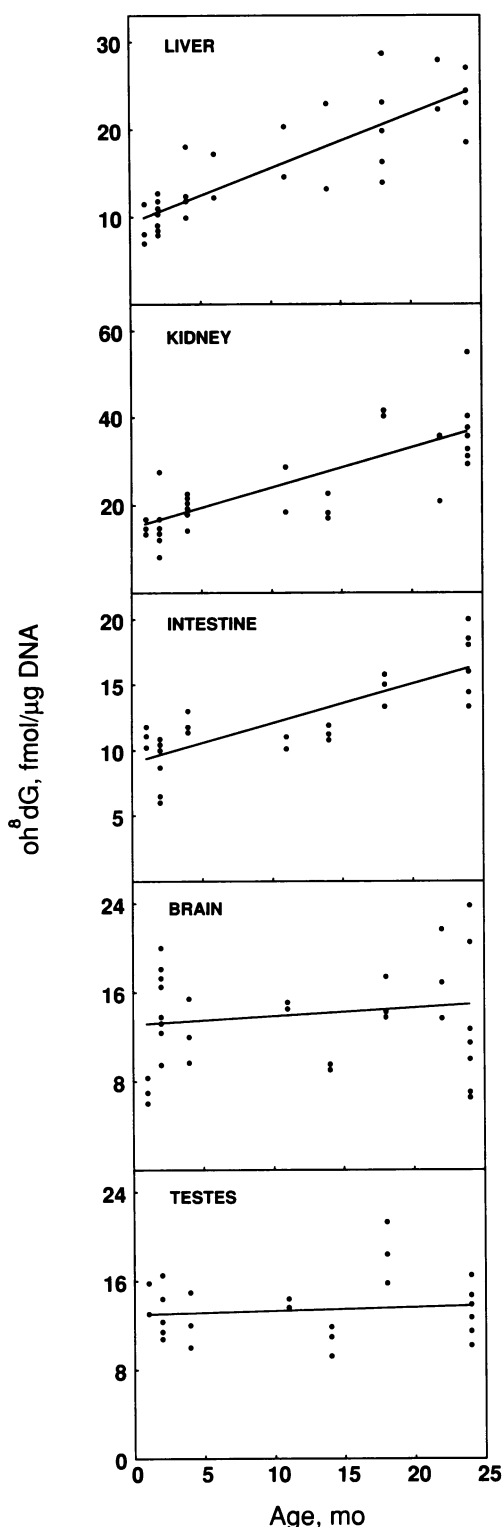


FIG. 1. Content of  $\text{oh}^8\text{dG}$  in DNA from organs of Fischer 344 rats as a function of age. Each point represents the average of at least two determinations for each animal. The lines were obtained by regression analysis;  $P < 0.05$  for liver, kidney, and intestine; not significant for brain and testes.

a glycosylase repair activity, but to obtain an accurate quantitative estimate it is important to control for diet.

### DISCUSSION

One of the molecular theories of aging is that DNA accumulates errors that shorten life-span (30–32). Damage to DNA from endogenous mutagens is likely to be critical for both

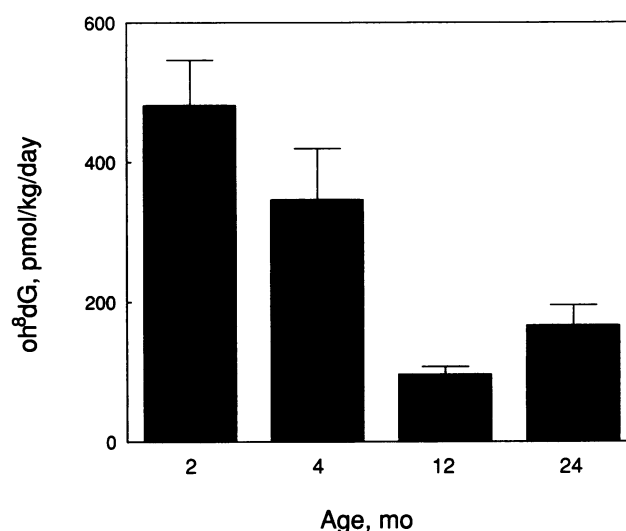


FIG. 2. Content of  $\text{oh}^8\text{dG}$  in urine excreted by Fischer 344 rats of different ages. Each value represents the average from three animals  $\pm$  SE (4 and 12 months) or six animals (2 and 24 months).

aging and cancer (7, 33). The free radical theory of aging suggests that the main endogenous mutagens are oxidants coming from partial oxygen reduction or products of lipid peroxidation (3). Consequently, the accumulation of free radical-mediated damage might be a major cause of the physiological changes associated with aging and cancer.

When DNA is oxidized *in vitro* (for example, by ionizing radiation), a number of damage products (e.g., base damage, sugar damage, and protein–DNA crosslinks) are formed (34, 35). One of these products,  $\text{oh}^8\text{dG}$ , has been proposed to be an indicator of oxidative damage in DNA both *in vitro* and *in vivo* (11, 21, 22). Estimates of  $\text{oh}^8\text{dG}$  yields from  $\gamma$ -irradiated DNA in aqueous solutions are 5% of the expected total damage to DNA (36). Therefore, we assume that the background levels of  $\text{oh}^8\text{dG}$  in DNA are likely to be an underestimate of the oxidative damage produced through normal aerobic metabolism by roughly a factor of 20, although the value cannot be calculated exactly.

The presence of  $\text{oh}^8\text{dG}$  in DNA isolated from five rat organs indicates that oxidative damage to DNA is a ubiquitous process occurring under normal physiological conditions. The number of modified residues per normal base (8–73  $\text{oh}^8\text{dG}$  per  $10^6$  deoxyguanosine residues) is extremely high compared to other reported adducts and confirms the steady-state levels reported previously for this oxidized adduct in DNA isolated from the livers of Sprague–Dawley rats (11). These high values would be a result of the continuous production of oxygen radicals by the unavoidable error rate of one electron addition to oxygen in the electron transport chain of mitochondria and other endogenous metabolic processes that utilize oxygen (4, 8).

The average content of  $\text{oh}^8\text{dG}$  in liver DNA from 4-month-old rats is 13 fmol per  $\mu\text{g}$  of DNA. From this value, we estimate a steady-state level in rat DNA of  $\approx 47,000$   $\text{oh}^8\text{dG}$  residues per cell, considering that mammalian cells contain 6 pg of DNA (37).

The formation of  $\text{oh}^8\text{dG}$  in DNA is expected to be a continuous process. We therefore expect, as with other DNA lesions, that this damage would be repaired to avoid its rapid and lethal accumulation. An endonuclease activity has been identified in *E. coli* that repairs  $\text{oh}^8\text{dG}$  from DNA [Chung, M.-H., Kasai, H., Jones, D., Ohtsuka, E. & Nishimura, S., 48th Meeting of the Japanese Cancer Association, October, 1989 (Abstr. 198)]. The measurement of  $\text{oh}^8\text{dG}$ , the expected product of an endonuclease activity, and  $\text{oh}^8\text{Gua}$ , the pro-

posed product of the  $\text{oh}^8\text{dG}$  glycosylase activity, can be used to estimate the total rate of repair of  $\text{oh}^8\text{dG}$  residues present in DNA. From the urinary excretion rate of  $\text{oh}^8\text{dG}$  in 4-month-old rats ( $1150 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ), we calculate that a rat excretes  $\approx 1035$   $\text{oh}^8\text{dG}$  residues per cell per day. [To calculate this value it is necessary to assume that the cell density in a rat is the same as in a human. Thus, the value of  $5 \times 10^{13}$  cells per 70-kg human (38) would be equivalent to  $2 \times 10^{11}$  cells per 300-g rat.] The excretion rate of  $\text{oh}^8\text{Gua}$  (corrected for diet) was significantly higher, averaging  $3647 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  or 3282 residues per cell per day. Consequently, if the contribution of diet toward the urinary levels of  $\text{oh}^8\text{Gua}$  is corrected for, then the  $\text{oh}^8\text{Gua}$  detected by our assay is due to the repair of  $\text{oh}^8\text{dG}$  residues in DNA by a glycosylase activity. Thus, we calculate from the urinary  $\text{oh}^8\text{Gua}$  values obtained from rats fed nucleic acid-free diets that the total rate of repair by the nuclease and glycosylase activities combined will be  $\approx 4317$  residues per cell per day.

The rate of accumulation of  $\text{oh}^8\text{dG}$  in DNA should reflect the relationship between the rates of its formation and repair. From the slope of the plot of the steady-state level of  $\text{oh}^8\text{dG}$  in rat kidney DNA as a function of age (Fig. 1), we calculate that each cell accumulates 80  $\text{oh}^8\text{dG}$  residues per day. This rate is significantly lower than the rate at which residues of the repair of  $\text{oh}^8\text{dG}$  are excreted into urine (4317 residues per cell per day), a result that leads us to conclude that the repair of  $\text{oh}^8\text{dG}$  residues is very efficient. We estimate that in kidney DNA for every 54 residues of  $\text{oh}^8\text{dG}$  repaired, 1 residue remains unrepaired. For the DNA isolated from other tissues, such as brain and testes, this rate of accumulation is substantially lower or nonexistent, reflecting even more efficient repair.

The rate at which  $\text{oh}^8\text{dG}$  residues are formed in DNA should be approximately equivalent to the sum of the rate of its accumulation and excretion. The rate of accumulation is small as compared with the rate of excretion; therefore, the rate of formation of  $\text{oh}^8\text{dG}$  residues in DNA will be roughly equivalent to its rate of repair (defined above as the sum of the excretion rates of  $\text{oh}^8\text{dG}$  and  $\text{oh}^8\text{Gua}$ ).

The formation of oxygen radicals, based on superoxide anion generation *in vitro* in rat lung and liver mitochondria and microsomes (8, 39, 40), is estimated to be about 50 nmol per g of tissues per min or  $6.5 \times 10^{10}$  oxygen radicals per cell per day. If we assume that the total amount of oxidized DNA residues is equivalent to the rate of formation of  $\text{oh}^8\text{dG}$  multiplied by the factor of 20 mentioned above, we can calculate that  $8.6 \times 10^4$  oxidized DNA residues are formed per cell per day. Comparing the amount of oxidized DNA residues with the number of oxygen radicals being produced, it is possible to estimate that one oxidized DNA residue is formed for every  $7.6 \times 10^5$  oxygen radicals generated through aerobic cellular metabolism.

Accumulation of oxidative DNA adducts with age could increase the level of spontaneous mutagenesis. Previously, age-associated increases in the levels of unidentified DNA adducts detected by electrochemical detection (41) and  $^{32}\text{P}$  postlabeling (42) have been reported, although the relationships between such adducts and aging remain to be understood. In the present report, we show that the steady-state level of  $\text{oh}^8\text{dG}$  increases steadily with age in DNA isolated from liver, kidney, and intestine of rats. Over the life-span of the rat, this accumulation amounts to roughly a 2-fold increase in the steady-state level of this oxidatively damaged product. This increase is more impressive if  $\text{oh}^8\text{dG}$  steady-state levels are corrected by the metabolic rate (rate of oxygen consumption), which decreases with the age of the animal.

Extrapolating to age zero, it is possible to estimate a high number of residues present at birth. It is likely that oxidative damage occurs during embryogenesis when the rate of cell

proliferation is high, and when a large portion of the genome is exposed to this type of damage. One possibility is that  $\text{oh}^8\text{dG}$  residues present in DNA during development are distributed throughout the whole genome, while its distribution during other periods of the life cycle is predisposed toward accumulation in portions of the genome that are not actively transcribed.

The increase in the steady-state level of  $\text{oh}^8\text{dG}$  in rat liver, kidney, and intestine could be associated with changes in the functions of these organs and would increase their susceptibility to tumor development. Age-associated changes in kidney function are extremely marked (43) and are also noticeable in the liver and the intestine (44). The high steady-state level of  $\text{oh}^8\text{dG}$  in kidney DNA is consistent with the fact that of the studied organs, it has the highest oxygen consumption per g of tissue (45). The constant value of the steady-state level of  $\text{oh}^8\text{dG}$  measured in testes during the life-span of the rat would indicate a very efficient repair system present in this tissue, which protects the reproductive organs from accumulating oxidative damage. In brain the observed results also suggest an efficient repair system. It could be argued that the three organs that showed increased steady-state levels of  $\text{oh}^8\text{dG}$  with age are organs that are directly exposed to oxidants and whose detoxification mechanisms are known to diminish with age (44) and that brain and testes have a blood barrier that limits their exposure to xenobiotics or endogenously produced mutagens. It would seem unlikely that exogenous oxidants in the standard rat diet are very important compared to the high rate of endogenous production of reactive oxygen species.

The age-dependent increase in the steady-state level of oxidized adducts of DNA would be the result of an increase in the formation of such adducts, a decrease in the repair activity, or a combination of both. A general increase in oxidative conditions with age is not supported by the decrease in metabolic rate occurring with age. The decrease of the urinary excretion of  $\text{oh}^8\text{dG}$  with age could be a consequence of an age-dependent decrease in the nuclease activities responsible for the repair of  $\text{oh}^8\text{dG}$ . It is premature to evaluate the effect of age on the urinary excretion of  $\text{oh}^8\text{Gua}$ , because of the major contribution of diet that has not been adequately corrected for. Further studies will need to be performed to understand the role of the putative  $\text{oh}^8\text{dG}$  glycosylase repair enzyme and its activity during aging. Our results would indicate either a decrease in the endonuclease activity or a combined effect of loss of repair and organ-specific increase in oxidative conditions. Age-dependent decreases in UV-induced DNA repair in mouse lymphocytes (46) and  $\text{O}^6$ -methylguanine transferase activities in mouse liver (47) have been reported. Concerning antioxidant defenses, the activity of superoxide dismutase, catalase, and glutathione peroxidase have been shown to decline with age in liver and kidney of female Wistar rats, but superoxide dismutase and glutathione peroxidase remained constant in brain (48).

This study shows that a specific product of endogenous oxidative damage to DNA,  $\text{oh}^8\text{dG}$ , which has been demonstrated to cause misreading of synthetic templates *in vitro* (49), is present in the DNA from different rat organs and increases with age in liver, kidney, and intestine but remains constant in brain and testes. This tissue specificity indicates that in complex organisms, oxidative stress does not affect all organs to the same extent. The steady-state accumulation of  $\text{oh}^8\text{dG}$  in the DNA of some of the organs examined is likely to increase the level of spontaneous mutagenesis, an event that may contribute to aging and other age-related pathologies such as cancer.

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